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CONTEMPORARY REVIEW

Mechanisms of Action Point Towards Combined PBDE/NDL-PCB Risk Assessment

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ABSTRACT

At present, human risk assessment of the structurally similar non-dioxin-like (NDL) PCBs and polybrominated diphenylethers (PBDEs) is done independently for both groups of compounds. There are however obvious similarities between NDL-PCBs and PBDEs with regard to modulation of the intracellular calcium homeostasis (basal calcium levels, voltage-gated calcium channels, calcium uptake, ryanodine receptor) and thyroid hormone (TH) homeostasis (TH levels and transport). which are mechanisms of action related to neurobehavioral effects (spontaneous activity, habituation and learning ability). There also similarities in agonistic interactions with the hepatic nuclear receptors PXR and CAR. Several effects on developmental (reproductive) processes have also been observed, but results were more dispersed and insufficient to compare both groups of compounds. The available mechanistic information is sufficient to warrant a dose addition model for NDL-PCBs and PBDEs, including their hydroxylated metabolites.
Although many of the observed effects are similar from a qualitative point of view for both groups, congener or tissue specific differences have also been found. As this is a source of uncertainty in the combined hazard and risk assessment of these compounds, molecular entities involved in the observed mechanisms and adverse outcomes associated with these compounds need to be identified. The systematical generation of (quantitative) structure-activity information for NDL-PCBs and PBDEs on these targets (including potential non-additive effects) will allow a more realistic risk estimation associated with combined exposure to both groups of compounds during early life. Additional validation studies are needed to quantify these uncertainties for risk assessment of NDL-PCBs and PBDEs.

Key words: NDL-PCBs; PBDEs; neurobehavior; calcium; thyroid; PXR/CAR.

Humans are commonly exposed to polychlorinated biphenyls (PCBs) and polybrominated diphenylethers (PBDEs). Common routes of exposure for humans are via ingestion of household dust, inhalation, dairy products, meat, fish and human milk. For PCBs as well as PBDEs, the highest intake is found for infants, toddlers, and small children, with intake decreasing with age and increasing body weight (El Majidi *et al.*, 2014; Frederiksen *et al.*, 2009). It is well established that prenatal and postnatal exposure via human milk exposes the neonate to both PCBs and PBDEs (UNEP, 2013).

PCBs have been widely used for industrial and commercial applications, such as heat transfer fluids, plasticizers, and flame-retardants (Safe, 1993). Brominated flame-retardants, such as PBDEs, were introduced in the 1970s as replacements of PCBs. PBDEs are used mainly as additive flame-retardants (not covalently bound to the polymers), resulting in leakage from consumer products into the environment (Birnbaum and Staskal, 2004). PCBs have been banned globally since the 1980s, while PBDEs have been banned in the EU during the last decade. Since these bans, exposure levels have been found to be declining (eg, Guo *et al.*, 2016; Shunthirasingham *et al.* 2016). Nevertheless, PCBs and PBDEs are still being detected in the environment and humans and expected to remain in the environment due to their persistence for decades to come. Based on the

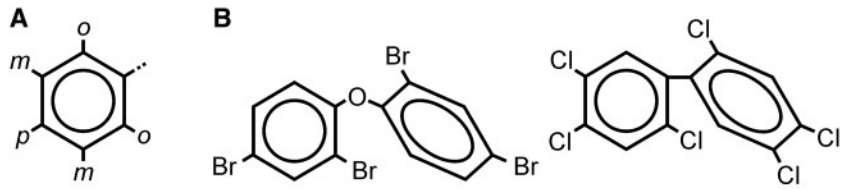

FIG. 1. A, Both PBDEs and (NDL-)PCBs consist of 2 interconnected benzene rings with one or more substitutions (bromine or chlorine, respectively) on meta (*m*), para (*p*), and/or ortho (*o*) positions. **B,** Molecular structures of PBDEs (eg, BDE-47; left) and (NDL-)PCBs (eg, PCB-153; right).

amount of exposure most PCBs to which humans are exposed do not exhibit dioxin-like (DL) properties and therefore usually called non dioxin-like (NDL) PCBs. NDL-PCBs and PBDEs included in this review are those that are predominantly detected in humans and wildlife. Moreover, hydroxylated metabolites of both NDL-PCBs and PBDEs have also been found in different human tissues and blood (Chen *et al.*, 2013; Quinete *et al.*, 2014).

At present, human risk assessment of PCBs is differentiated with, on the one hand, the sum of PCB congeners that exhibit DL properties [on a relative effect potency (REP) basis; Van den Berg *et al.* 2006] or on the other hand, the total sum of all PCB congeners. The human risk assessment of PBDEs, based on individual congeners or total sum of PBDE congeners, has so far been done independently of that for PCBs. This is in spite of the fact that environmentally common PBDEs bear a strong structural similarity with NDL-PCBs (Figure 1).

In this review we evaluate and discuss the aptness of combining exposure and effects of NDL-PCBs and PBDEs in human risk assessment based on mechanistic considerations. To this aim, experimental endpoints by NDL-PCBs and PBDEs and their hydroxylated metabolites are discussed in relation to mechanisms of action (Table 1) with special focus on exposure and effects during early life stages. From a mixture toxicity point of view we also focus on the potential additive effects based on mechanisms of action of both groups of compounds.

METHODOLOGY

Literature searches for peer-reviewed articles in the scientific literature addressing toxicity and biological effects of NDL-PCBs and/or PBDEs in experimental *in vitro* and *in vivo* mammalian models were performed using the PubMed database of the US National Library of Medicine. NDL-PCB and PBDE congeners that are commonly detected in human milk have been included in this review based on global surveys from the World Health Organization and UNEP (UNEP, 2013). These surveys provide a clear and timely overview of the quantitative exposure of human newborns as well as the maternal body burden. In addition, the levels of PCBs and PBDEs in human milk can be used as a proxy for regional exposures and support further remedial actions for these persistent organic pollutants. Data on cytotoxicity and related mechanisms were not included. It was accepted that NDL-PCBs and PBDEs are not able to significantly activate the aryl hydrocarbon receptor AhR and induce specific DL toxicity and biological effects, eg, CYP1A1 induction (Peters *et al.*, 2004). Therefore, experimental studies in which PCBs or PBDEs induced CYP1A1 activity were excluded from this review as this may indicate the presence of contaminating DL compounds, which may obscure specific effects of NDL-PCBs and PBDEs. We also excluded experimental studies if commercial PCB or PBDE mixtures such as Aroclor-1254 and DE-71 were used, because their contamination with DL compounds is well established.

NDL-PCBs AND PBDEs: EFFECT SIMILARITIES

Neurobehavior

Neurobehavioral effects resulting from exposure to NDL-PCBs and PBDEs, in particular after pre- and/or postnatal exposure, have been studied extensively in rodents. Common endpoints included tests for locomotor activity, spontaneous behavior, habituation capability, spatial learning and anxiety, which were measured in open-field set-ups and Morris water maze or radial arm mazes.

Although some studies found reduced spontaneous activity following exposure to PBDEs (Ta *et al.*, 2011; Viberg *et al.*, 2006; Zhang *et al.*, 2013) and NDL-PCBs (Boix *et al.*, 2011), most studies in mice and rats perinatally exposed to NDL-PCBs as well as PBDEs showed an increase in spontaneous activity (Gee and Moser, 2008; Gralewicz *et al.*, 2009; Holene *et al.*, 1998; Kuriyama *et al.*, 2005; Lesmana *et al.*, 2014; Suvorov *et al.*, 2009). Neurobehavioral studies with mice that have used different PBDE and NDL-PCB congeners have also reported a reduced habituation capability for both groups of compounds (Eriksson *et al.*, 2001; Eriksson and Fredriksson, 1996; Johansson *et al.*, 2008; Viberg *et al.*, 2003a,b, 2004) as well as impaired spatial learning and memory abilities in both rats and mice (Boix *et al.*, 2010; Piedrafita *et al.*, 2008; Schantz *et al.*, 1995; Yan *et al.*, 2012).

From a quantitative point of view, it should be noted that these neurobehavioral studies with NDL-PCBs and PBDEs often involved a limited number of dose levels. This precludes the determination of REPs due to the lack of complete dose-response relationships. In addition, the lowest observed (adverse) effect levels vary strongly between congeners and measured endpoints and may differ as much as 3 orders of magnitude. So far, few studies investigated the effects of NDL-PCBs or PBDEs on specific neurotransmitter systems after *in vivo* (perinatal) exposure, but some interactions have been observed that relate to the cholinergic, serotonergic and dopaminergic systems (Castoldi *et al.*, 2006; Coccini *et al.*, 2011; Honma *et al.*, 2009; Lilienthal *et al.*, 2014; Seegal *et al.*, 1997; Viberg *et al.*, 2002). Mechanism(s) of action for these neurobehavioral effects cannot be established based on these data alone, and various cellular and molecular processes may underlie the observed effects, which could hamper the estimation of neurotoxicity risk resulting from combined exposure to PBDEs or NDL-PCBs. In Table 1, an overview is presented of effects observed in behavioral studies with specific NDL-PCBs and PBDEs or hydroxy-metabolites.

Calcium Homeostasis

Calcium (Ca^{2+}) is involved in numerous cellular and subcellular neuronal processes, such as neurotransmitter release by exocytosis, cell death, and mitochondrial function. Calcium release from the endoplasmic reticulum, in particular via ryanodine

TABLE 1. Overview of affected endpoints (health effects and mechanisms of action) by NDL-PCBs and PBDEs and their hydroxylated metabolites with regard to neurobehavior (A), calcium homeostasis and signaling (B), the thyroid system (C) and CYP induction via PXR/CAR (D) as observed in experimental studies.

		PBDEs	PCBs
A	spontaneous behavior (*in vivo*)	Gee and Moser, 2008 Kuriyama *et al.*, 2005 Suvorov *et al.*, 2009 Ta *et al.*, 2011 Viberg et al., 2006 Zhang et al., 2013	Boix et al., 2011 Gralewicz et al., 2009 Holene et al., 1998 Lesmana et al., 2014
	habituation capability (*in vivo*)	Eriksson et al., 2001 Johansson et al., 2008 Viberg et al., 2003a Viberg et al., 2003b Viberg et al., 2004	Eriksson and Fredriksson, 1996
	spatial learning (*in vivo*)	Ta et al., 2011 Yan et al., 2012	Boix et al., 2010 Piedrafita et al., 2008 Schantz et al., 1995
B	basal intracellular calcium concentration (*in vitro*)	Coburn et al., 2008 Dingemans et al., 2007 Dingemans et al., 2008 Dingemans et al., 2010a Gassmann et al., 2014 He et al., 2009 Kodavanti et al., 1996 Pereira et al., 2013	He et al., 2009 Johansson et al., 2006 Llansola et al., 2010 Tan et al., 2004 Yilmaz et al., 2006
	depolarization-evoked calcium concentration (*in vitro*)	Dingemans et al., 2010b	Langeveld et al., 2012
	ryanodine receptor activation (*in vitro*)	Kim et al., 2011	Pessah et al., 2006 Pessah et al., 2010
	kinase signaling (*in vitro*)	Fan et al., 2010 Li et al., 2013	Fan et al., 2010 Lee and Yang, 2012
C	thyroxin levels in blood and plasma (*in vivo*)	Blanco et al., 2013 Kuriyama et al., 2007 Richardson et al., 2008	Desaulniers et al., 1999 Hedge et al., 2009 Kato et al., 2010 Liu et al., 2012 Meerts et al., 2002
	triiodothyronine levels in blood and plasma (*in vivo*)	Blanco et al., 2013 Lee et al., 2010	Kato et al., 2011 Liu et al., 2012
	TH receptor binding (*in vitro*)	Li et al., 2010 Ren et al., 2013	Kitamura et al., 2005
	TH binding to TTR (*in vitro*)	Cao et al., 2010 Marchesini et al., 2008 Meerts et al., 2000	Chauhan et al., 2000 Lans et al., 1993 Lans et al., 1994 Marchesini et al., 2008 Purkey et al., 2004
	TH binding to TBG (*in vitro*)	Cao et al., 2010 Marchesini et al., 2008	Lans et al., 1994 Marchesini et al., 2008
D	PXR activation (*in vivo* and *in vitro*)	Pacyniak et al., 2007	Al-Salman and Plant, 2012 Gährs et al., 2013 Kopec et al., 2010
	CAR activation (*in vivo* and *in vitro*)	Lee et al., 2010 Sueyoshi et al., 2014	Al-Salman and Plant, 2012 Gährs et al., 2013 Kamata et al., 2015 Kopec et al., 2010

Abbreviations: TBG, thyroxine-binding globulin; TTR, transthyretin.

receptors or 1,4,5-triphosphate receptors, plays a role in the modulation of the intracellular calcium signals (Berridge, 2012). In this respect, it is important to note that for a number of NDL-PCBs and PBDEs congeners similar effects on calcium homeostasis and signaling were detected in several studies with cell lines and primary cells.

Increased basal intracellular Ca^{2+} levels were most commonly observed following exposure to different NDL-PCB or PBDE congeners (He *et al.*, 2009; Tan *et al.*, 2004; Yilmaz *et al.*, 2006), but in a few studies, a decrease of basal intracellular Ca^{2+} levels was observed (Llansola *et al.*, 2010). Disruption (increase) of basal $[Ca^{2+}]_i$ can be caused by an impairment of the

uptake of Ca^{2+} by organelles or an increase of the efflux of Ca^{2+} from organelles (besides influx of extracellular Ca^{2+}), and both mechanisms were detected following exposure to PBDE as well as NDL-PCB congeners (Coburn *et al.*, 2008; Johansson *et al.*, 2006; Pereira *et al.*, 2013). Influence of the molecular structure is observed [eg, NDL-PCBs with chlorine atoms at the *ortho*- and *ortho-lateral (meta, para)* positions were most potent for their effects on Ca^{2+} uptake in organelles (Kodavanti *et al.*, 1996)] but this is not clearly observed in all studies.

For PBDEs, *in vitro*, the hydroxylated metabolite 6-OH-BDE-47 was at least one order of magnitude more potent than its parent compound in increasing basal Ca^{2+} levels (Dingemans *et al.*, 2007, 2008). In a following up structure-activity study, this increased potency was confirmed for other hydroxylated PBDEs while higher brominated PBDEs did not affect basal Ca^{2+} levels. It was demonstrated in this study that shielding of the OH group by adjacent bromine atoms and/or the proximity of the ether bond lowers the higher potency of these metabolites relative to the PBDE parent compounds (Dingemans *et al.*, 2010a). Comparable effects of 6-OH-BDE-47 on basal Ca^{2+} cellular levels were also observed in human neuroprogenitor cells at relatively low concentrations (0.2 μM; Gassmann *et al.*, 2014).

Exposure to NDL-PCBs and OH-PBDEs has also been show to inhibit $[Ca^{2+}]_i$ increases evoked *in vitro* by depolarization (Dingemans *et al.*, 2010b; Langeveld *et al.*, 2012). Few effects were observed for PBDE parent congeners, while inhibition of depolarization-evoked Ca^{2+} increases by OH-PBDEs was mostly associated with preceding increases in basal Ca^{2+} due to Ca^{2+} release from intracellular stores (Dingemans *et al.*, 2010b). A structure-activity study showed that tri-, tetra-, and some pentachlorinated NDL-PCB congeners disturbed the calcium homeostasis while NDL-PCBs with 6 or more chlorines showed no or only minor effects on basal and depolarization-evoked Ca^{2+} levels (Langeveld *et al.*, 2012).

Both NDL-PCBs and PBDEs as well as their hydroxylated metabolites are also modulators of RyR activation, which is another mechanism of action causing disruption of Ca^{2+} homeostasis and signaling (Pessah *et al.*, 2010). A structure-activity study that focused on the potency of NDL-PCBs towards sensitizing RyR1 receptor activation again showed the importance of *ortho* and *meta* chlorine substitutions and metabolic hydroxylation of these PCBs increases the activity towards RyR (Pessah *et al.*, 2006). *Ortho*- and (absence of) *para* bromine substitutions are also critical for such effects by PBDEs and effects of OH-PBDEs on RyR activation (Kim *et al.*, 2011).

In summary, there are clear similarities in mechanisms of actions of PBDEs and NDL-PCBs as well as their hydroxylated metabolites in disturbing calcium homeostasis via release of Ca^{2+} from intracellular stores and inhibition of Ca^{2+} signaling (for overview see Table 1). This is cause for concern with regard to combined exposure, as disruption of the calcium homeostasis in (developing) neuronal cells could be one of the fundamental causes of neurobehavioral effects observed later in life.

Although the small number of studies on these phenomena currently precludes a detailed comparison between both groups of compounds, there are numerous other mechanisms of actions observed of PBDEs and/or NDL-PCBs that might underlie the observed neurobehavioral effects. NDL-PCBs and PBDEs both affect calcium-related kinase and pathways, which are directly involved in the modulation of release of calcium from intracellular stores (e.g. Fan *et al.*, 2010; Lee and Yang, 2012). Furthermore, PBDEs affect neurite outgrowth and neuronal migration (Schreiber *et al.*, 2009; Xiong *et al.*, 2012) and congeners from both groups of compounds have been shown to affect the cytoskeleton in neurons (Alm *et al.*, 2008; Brunelli *et al.*, 2012) and modulate neurotransmitter receptor function (Westerink, 2014).

Thyroid System

Thyroid hormones (THs) are essential for growth and development in the fetal and post-natal stage, but also regulate metabolism in adults (Brent, 2012). THs are also essential for development of the nervous system (Schroeder and Privalsky, 2014).

Multiple studies have shown that NDL-PCBs, PBDEs and their hydroxylated metabolites can all significantly influence the TH homeostasis (Lans *et al.*, 1994; Meerts *et al.*, 2000; Murk *et al.*, 2013). Although results are not consistent, most *in vivo* studies showed a decrease in total and free thyroxin (T_4) concentrations following exposure to NDL-PCBs or PBDEs (Blanco *et al.*, 2013; Hedge *et al.*, 2009; Kato *et al.*, 2010; Kuriyama *et al.*, 2007; Liu *et al.*, 2012; Richardson *et al.*, 2008). Accumulation of T_4 in the liver (Kato *et al.*, 2011) and an increase in T_4 glucuronidation have been suggested as responsible mechanism for these observations (Richardson *et al.*, 2014). However, there are also several *in vivo* studies that did not observe alterations in free and total T_4 concentrations after NDL-PCB and PBDE exposure (Gee *et al.*, 2008; Kobayashi *et al.*, 2009; Tseng *et al.*, 2008). Although an adequate explanation for this discrepancy is lacking, it may be related to differences in dose levels and/or adaptive mechanism(s) during the exposure window.

Another mechanism is the inhibition of competitive binding of T_4 to transport proteins transthyretin (TTR) and thyroxin-binding globulin (TBG), which has been observed for hydroxylated metabolites of both (NDL-) PCBs and PBDEs (Cao *et al.*, 2010; Marchesini *et al.*, 2008; Meerts *et al.*, 2000, 2002; Purkey *et al.*, 2004). Structure-activity and *in vitro* studies with NDL-PCBs, PBDEs and their hydroxylated -metabolites showed that the position of the OH-group and presence of adjacent bromines play an important role in the binding affinity to the TH receptor and transport proteins (Chauhan *et al.*, 2000; Kitamura *et al.*, 2005; Lans *et al.*, 1993; Li *et al.*, 2010; Ren *et al.*, 2013; Yang *et al.*, 2011). *In vivo* studies that address the role of hydroxylated metabolites of PCBs and PBDEs are scarce, but one study using prenatal exposure to OH-PCB-107 in rats reported a decrease in total and free T_4 in the offspring (Meerts *et al.*, 2002). Further studies are warranted to investigate whether these hydroxylated metabolites of NDL-PCBs and PBDEs may indeed exert stronger *in vivo* effects than their parent compounds.

There are also a number of *in vivo* studies in which a decrease in serum triiodothyronine (T_3) concentrations has been observed following exposure to PBDEs (eg, Blanco *et al.*, 2013; Lee *et al.*, 2010) or NDL-PCBs (Kato *et al.*, 2011; Liu *et al.*, 2012), but again there are studies that could not find any effect on T_3 levels (eg, He *et al.*, 2011; Kato *et al.*, 2012; Ness *et al.*, 1993). In contrast, increases in T_3 or T_4 levels have been rarely reported (Desaulniers *et al.*, 1999). Although several studies have investigated a possible effect on levels of thyroid-stimulating hormone (TSH), no significant effect has been reported for either group of compounds (He *et al.*, 2011; Kato *et al.*, 2011).

In Table 1 an overview is presented of effects of NDL-PCBs and PBDEs or hydroxy-metabolites on the thyroid system. As imbalance of TH homeostasis in the early life stage can result in lower cognitive functions later in life, the similarities in effects of PBDEs and NDL-PCBs on this endocrine system are of concern, in particular with regard to combined exposure.

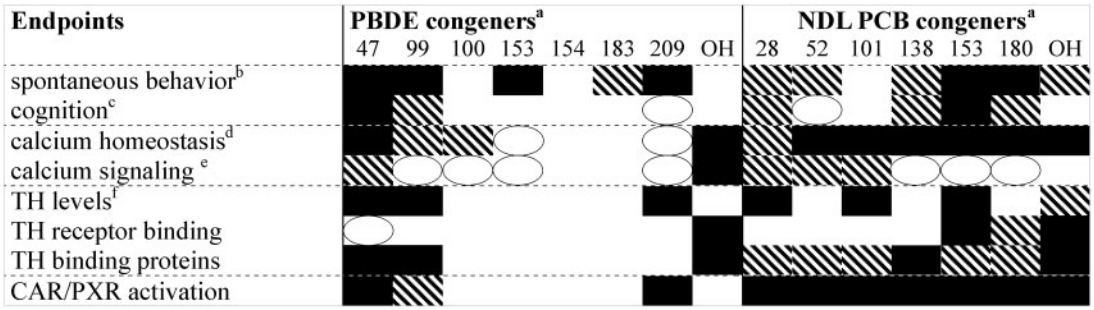

FIG. 2. Schematic overview of similarities in effects of NDL-PCBs and PBDEs. A solid fill indicates that there are multiple studies that have demonstrated the effect of a congener on a particular endpoint, while a broken fill indicates that the evidence is derived from an individual study. A clear ellipse indicates that there is experimental evidence in the literature demonstrating a lack of effect of a congener on a particular endpoint. Further evaluation is warranted to identify the most appropriate studies for the establishment of REPs. Notes: a. IUPAC congener naming system; b. effects observed on measures of spontaneous motor activity; c. effects observed in cognitive tests for spatial and task learning; d. effects observed on basal Ca^{2+} levels, uptake of Ca^{2+} in organelles and RyR activation; e. effects observed on depolarization-evoked Ca^{2+}; f. effects on T_4, T_3, and/or TSH levels.

Activation of PXR and CAR

The constitutive androstane (CAR) and pregnane X (PXR) receptors belong to the same nuclear hormone receptor family and are expressed in liver and intestine (Kretschmer and Baldwin, 2005). Both receptors regulate several CYP enzymes (eg, CYP2B and CYP3A), conjugating enzymes and transport proteins (eg, MDR1). Interactions of xenobiotics with PXR and CAR can thus influence metabolism and body distribution of exogenous and endogenous compounds such as pharmaceuticals and steroid hormones (Tolson and Wang, 2010). Furthermore, chronic activation of PXR and CAR is associated with adverse health effects, such as metabolic dysfunction, change in hormone metabolism and development or progression of various types of cancer (Banerjee *et al.*, 2015; Kretschmer and Baldwin, 2005).

NDL-PCBs can directly activate PXR and CAR resulting in subsequent transcription of target genes (eg, CYP3A4 and MDR1) at levels approximating human serum levels (Al-Salman and Plant, 2012; Gährs *et al.*, 2013; Kamata *et al.*, 2015; Kopec *et al.*, 2010). *In vitro* experiments with PBDEs also showed that these compounds are able to activate the PXR receptor, which resulted in induction of CYP3A11 and CYP2B10 (Pacyniak *et al.*, 2007). Another study established that PBDEs can also act as CAR agonists and induce the expression of CYP2B and CYP3A genes (Sueyoshi *et al.*, 2014). This CYP induction was also observed in BDE-209 exposed mice (Lee *et al.*, 2010). Taken together, several studies clearly showed that both NDL-PCBs and PBDEs are PXR and CAR agonists (Table 1), which at least results in CYP enzyme induction, which is also supported by the conclusions of a molecular modeling study (Wu *et al.*, 2009). Especially NDL-PCBs and PBDEs that contain multiple *ortho*-substitutions can have an agonistic effect on these nuclear receptors, suggesting that this position of a bromine or chlorine atom is important for binding to the PXR or CAR and subsequent gene expression. As both PBDEs and NDL-PCBs directly activate these receptors, the effects of combined exposure of these groups of compounds on excretion patterns of pharmaceuticals and hormone levels may be influenced and warrants further investigation.

SUMMARY OF EFFECTS

Similarities between effects of NDL-PCBs and PBDEs are in particular observed on neurobehavior, cellular calcium homeostasis, TH levels, and interactions with CAR/PXR. From a qualitative point of view the neurobehavioral observations were not always consistent, which may be caused by differences with regard to the experimental models and neurobehavioral endpoints between laboratories. The effects of both groups of compounds on calcium and TH homeostasis are predominantly in the same direction, with hydroxylated-metabolites of NDL-PCBs and PBDEs being significantly more active than the parent compounds. Agonistic interactions with PXR and CAR of NDL-PCBs and PBDEs also show structural similarities with *ortho* chlorine or bromine substitutions being a major determinant for this effect. It should also be noted that there are also some (*in vivo*) studies in which specific effects of specific PBDEs or NDL-PCBs on neurobehavior or the thyroid system could not be demonstrated (eg, Boix *et al.* 2011; Gee *et al.* 2008). These apparent discrepancies with many of the studies mentioned earlier may be attributed to specific mechanisms (eg, metabolism) or the limited number of dose levels studied. In Figure 2a schematic overview is given for the above mentioned effects of NDL-PCBs and PBDEs, which indicates overall similarities in mechanisms of action between both groups of compounds.

FUTURE DIRECTIONS

Based on the majority of the available data on mechanisms of action, a concentration/dose-addition model appears likely for the hazard and risk assessment of NDL-PCBs and PBDEs, as suggested earlier by (Simon *et al.*, 2007; Westerink, 2014) for neurotoxicity. Nevertheless, a number of uncertainties and information gaps need to be identified and resolved.

The likeliness for (non) additive effects or mechanistic interactions between NDL-PCBs and PBDEs (eg, Eriksson *et al.*, 2006; He *et al.*, 2009, 2011) and differences in REPs generate uncertainty in hazard and risk assessment. Part of this uncertainty may be resolved if the molecular entities involved in adverse health outcomes are identified and quantified with regard to their associations with individual congeners of both groups of compounds and their hydroxylated metabolites. Uncertainties also remain with regard to the causal relations between the observed mechanisms of action and adverse health effects, in particular as it cannot be excluded that multiple mechanisms of action converge to result in health effects.

The use of *in vitro* models can help elucidate mechanisms of action that underlie possible (non) additive interactions of NDL-PCBs and PBDEs. Quantitative structure-activity and *in vitro* studies can be used for the future development of mechanism-specific toxic equivalent factor (TEF) values, in line with the present TEF concept for risk assessment of DL compounds (Van

den Berg *et al.*, 2006). In such a novel approach the hydroxylated metabolites of both NDL-PCBs and PBDEs should also be included, as their potency appears to be similar or even higher compared with that of their parent chemicals for some endpoints like the thyroid system, calcium homeostasis and steroidogenesis (Cantón *et al.*, 2006; Dingemans *et al.*, 2008; Meerts *et al.*, 2000). Preliminary TEF schemes for PCBs have already been developed based on *in vitro* neurotoxicity data (Simon *et al.*, 2007) and TH levels (Yang *et al.*, 2010). Such approaches can be expanded for both groups of compounds and newly identified molecular targets (eg, voltage gated calcium channels). A similar approach may be applied for the effects of NDL-PCBs and PBDEs or their metabolites on individual receptors such as the RyR, TR, PXR, or CAR. Furthermore, there is a clear need for adequate review of existing studies on which TEF values are based and experimental validation of such TEF values with *in vivo* studies to further improve the possibilities for incorporation in human risk assessment, especially for the early life stages.

The mechanistic information evaluated in this review to our opinion provides enough arguments to use additivity as an *interim* default approach for both groups of compounds in the risk assessment. In addition, *in vitro* studies are available to establish *interim* REPs for the quantitatively most important NDL-PCBs and PBDEs. Therefore we propose to combine *interim* REPs for both NDL-PCBs and PBDEs from *in vitro* studies in an additive manner. These can be related to human tissue levels used in a risk assessment framework to improve the estimation of risks associated with combined exposure to both groups of compounds.

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REFERENCES

Alm, H., Kultima, K., Scholz, B., Nilsson, A., Andrén, P. E., Fex-Svenningsen, A., Dencker, L., and Stigson, M. (2008). Exposure to brominated flame retardant PBDE-99 affects cytoskeletal protein expression in the neonatal mouse cerebral cortex. *Neurotoxicology* **29**, 628–637.

Al-Salman, F., and Plant, N. (2012). Non-coplanar polychlorinated biphenyls (PCBs) are direct agonists for the human pregnane-X receptor and constitutive androstane receptor, and activate target gene expression in a tissue-specific manner. *Toxicol. Appl. Pharmacol.* **263**, 7–13.

Banerjee, M., Robbins, D., and Chen, T. (2015). Targeting xenobiotic receptors PXR and CAR in human diseases. *Drug Discov. Today* **20**, 618–628.

Berridge, M. J. (2012). Calcium signalling remodelling and disease. *Biochem. Soc. Trans.* **40**, 297–309.

Birnbaum, L. S., and Staskal, D. F. (2004). Brominated flame retardants: Cause for concern? *Environ. Health Perspect.* **112**, 9–17.

Blanco, J., Mulero, M., Heredia, L., Pujol, A., Domingo, J. L., and Sánchez, D. J. (2013). Perinatal exposure to BDE-99 causes learning disorders and decreases serum thyroid hormone levels and BDNF gene expression in hippocampus in rat offspring. *Toxicology* **308**, 122–128.

Boix, J., Cauli, O., and Felipo, V. (2010). Developmental exposure to polychlorinated biphenyls 52, 138 or 180 affects differentially learning or motor coordination in adult rats. Mechanisms involved. *Neuroscience* **167**, 994–1003.

Boix, J., Cauli, O., Leslie, H., and Felipo, V. (2011). Differential long-term effects of developmental exposure to polychlorinated biphenyls 52, 138 or 180 on motor activity and neurotransmission. Gender dependence and mechanisms involved. *Neurochem. Int.* **58**, 69–77.

Brent, G. A. (2012). Mechanisms of thyroid hormone action. *J. Clin. Invest.* **122**, 3035–3043.

Brunelli, L., Llansola, M., Felipo, V., Campagna, R., Airoldi, L., De Paola, M., Fanelli, R., Mariani, A., Mazzoletti, M., and Pastorelli, R. (2012). Insight into the neuroproteomics effects of the food-contaminant non-dioxin like polychlorinated biphenyls. *J. Proteomics* **75**, 2417–2430.

Cantón, R. F., Sanderson, J. T., Nijmeijer, S., Bergman, A., Letcher, R. J., and van den Berg, M. (2006). *In vitro* effects of brominated flame retardants and metabolites on CYP17 catalytic activity: A novel mechanism of action? *Toxicol. Appl. Pharmacol.* **216**, 274–281.

Cao, J., Lin, Y., Guo, L. H., Zhang, A. Q., Wei, Y., and Yang, Y. (2010). Structure-based investigation on the binding interaction of hydroxylated polybrominated diphenyl ethers with thyroxine transport proteins. *Toxicology* **277**, 20–28.

Castoldi, A. F., Blandini, F., Randine, G., Samuele, A., Manzo, L., and Coccini, T. (2006). Brain monoaminergic neurotransmission parameters in weanling rats after perinatal exposure to methylmercury and 2,2',4,4',5,5'-hexachlorobiphenyl (PCB153). *Brain Res.* **1112**, 91–98.

Chauhan, K. R., Kodavanti, P. R., and McKinney, J. D. (2000). Assessing the role of ortho-substitution on polychlorinated biphenyl binding to transthyretin, a thyroxine transport protein. *Toxicol. Appl. Pharmacol.* **162**, 10–21.

Chen, A., Park, J. S., Linderholm, L., Rhee, A., Petreas, M., DeFranco, E. A., Dietrich, K. N., and Ho, S. M. (2013). Hydroxylated polybrominated diphenyl ethers in paired maternal and cord sera. *Environ. Sci. Technol.* **47**, 3902–3908.

Coburn, C. G., Currás-Collazo, M. C., and Kodavanti, P. R. (2008). *In vitro* effects of environmentally relevant polybrominated diphenyl ether (PBDE) congeners on calcium buffering mechanisms in rat brain. *Neurochem. Res.* **33**, 355–364.

Coccini, T., Roda, E., Castoldi, A. F., Poli, D., Goldoni, M., Vettori, M. V., Mutti, A., and Manzo, L. (2011). Developmental exposure to methylmercury and 2,2',4,4',5,5'-hexachlorobiphenyl (PCB153) affects cerebral dopamine D1-like and D2-like receptors of weanling and pubertal rats. *Arch. Toxicol.* **85**, 1281–1294.

Desaulniers, D., Leingartner, K., Wade, M., Fintelman, E., Yagminas, A., and Foster, W. G. (1999). Effects of acute exposure to PCBs 126 and 153 on anterior pituitary and thyroid hormones and FSH isoforms in adult Sprague Dawley male rats. *Toxicol. Sci.* **47**, 158–169.

Dingemans, M. M. L., Heusinkveld, H. J., Bergman, A., van den Berg, M., and Westerink, R. H. S. (2010a). Bromination pattern of hydroxylated metabolites of BDE-47 affects their potency to release calcium from intracellular stores in PC12 cells. *Environ. Health Perspect.* **118**, 519–525.

Dingemans, M. M. L., van den Berg, M., Bergman, A., and Westerink, R. H. S. (2010b). Calcium-related processes involved in the inhibition of depolarization-evoked calcium increase by hydroxylated PBDEs in PC12 cells. *Toxicol. Sci.* **114**, 302–309.

Dingemans, M. M. L., de Groot, A., van Kleef, R. G. D. M., Bergman, A., van den Berg, M., Vijverberg, H. P., and Westerink, R. H. S. (2008). Hydroxylation increases the neurotoxic potential of BDE-47 to affect exocytosis and calcium homeostasis in PC12 cells. *Environ. Health Perspect* **116**, 637–643.

Dingemans, M. M. L., Ramakers, G. M., Gardoni, F., van Kleef, R. G. D. M., Bergman, A., Di Luca, M., van den Berg, M., Westerink, R. H. S., and Vijverberg, H. P. (2007). Neonatal exposure to brominated flame retardant BDE-47 reduces long-term potentiation and postsynaptic protein levels in mouse hippocampus. *Environ. Health Perspect.* **115**, 865–870.

El Majidi, N., Bouchard, M., and Carrier, G. (2014). Systematic analysis of the relationship between standardized biological levels of polychlorinated biphenyls and thyroid function in pregnant women and newborns. *Chemosphere* **98**, 1–17.

Eriksson, P., Jakobsson, E., and Fredriksson, A. (2001). Brominated flame retardants: A novel class of developmental neurotoxicants in our environment? *Environ. Health Perspect.* **109**, 903–908.

Eriksson, P., Fischer, C., and Fredriksson, A. (2006). Polybrominated diphenyl ethers, a group of brominated flame retardants, can interact with polychlorinated biphenyls in enhancing developmental neurobehavioral defects. *Toxicol. Sci.* **94**, 302–309.

Eriksson, P., and Fredriksson, A. (1996). Developmental neurotoxicity of four *ortho*-substituted polychlorinated biphenyls in the neonatal mouse. *Environ. Toxicol. Pharmacol.* **1**, 155–165.

Fan, C. Y., Besas, J., and Kodavanti, P. R. (2010). Changes in mitogen-activated protein kinase in cerebellar granule neurons by polybrominated diphenyl ethers and polychlorinated biphenyls. *Toxicol. Appl. Pharmacol.* **245**, 1–8.

Frederiksen, M., Vorkamp, K., Thomsen, M., and Knudsen, L. E. (2009). Human internal and external exposure to PBDEs–A review of levels and sources. *Int. J. Hyg. Environ. Health* **212**, 109–134.

Gährs, M., Roos, R., Andersson, P. L., and Schrenk, D. (2013). Role of the nuclear xenobiotic receptors CAR and PXR in induction of cytochromes P450 by non-dioxinlike polychlorinated biphenyls in cultured rat hepatocytes. *Toxicol. Appl. Pharmacol.* **272**, 77–85.

Gassmann, K., Schreiber, T., Dingemans, M. M. L., Krause, G., Roderigo, C., Giersiefer, S., Schuwald, J., Moors, M., Unfried, K., Bergman, A., *et al.* (2014). BDE-47 and 6-OH-BDE-47 modulate calcium homeostasis in primary fetal human neural progenitor cells via ryanodine receptor-independent mechanisms. *Arch. Toxicol.* **88**, 1537–1548.

Gee, J. R., Hedge, J. M., and Moser, V. C. (2008). Lack of alterations in thyroid hormones following exposure to polybrominated diphenyl ether 47 during a period of rapid brain development in mice. *Drug Chem. Toxicol.* **31**, 245–254.

Gee, J. R., and Moser, V. C. (2008). Acute postnatal exposure to brominated diphenylether 47 delays neuromotor ontogeny and alters motor activity in mice. *Neurotoxicol. Teratol* **30**, 79–87.

Gralewicz, S., Wiaderna, D., Lutz, P., and Sitarek, K. (2009). Neurobehavioural functions in adult progeny of rat mothers exposed to methylmercury or 2,2', 4,4', 5,5'-hexachlorobiphenyl (PCB 153) alone or their combination during gestation and lactation. *Int. J. Occup. Med. Environ. Health* **22**, 277–291.

Guo, W., Holden, A., Smith, S. C., Gephart, R., Petreas, M., and Park, J. S. (2016). PBDE levels in breast milk are decreasing in California. *Chemosphere* **150**, 505–513.

He, P., Wang, A. G., Xia, T., Gao, P., Niu, Q., Guo, L. J., Xu, B. Y., and Chen, X. M. (2009). Mechanism of the neurotoxic effect of PBDE-47 and interaction of PBDE-47 and PCB153 in enhancing toxicity in SH-SY5Y cells. *Neurotoxicology* **30**, 10–15.

He, P., Wang, A., Niu, Q., Guo, L., Xia, T., and Chen, X. (2011). Toxic effect of PBDE-47 on thyroid development, learning, and memory, and the interaction between PBDE-47 and PCB153 that enhances toxicity in rats. *Toxicol. Ind. Health* **27**, 279–288.

Hedge, J. M., DeVito, M. J., and Crofton, K. M. (2009). *In vivo* acute exposure to polychlorinated biphenyls: Effects on free and total thyroxine in rats. *Int. J. Toxicol.* **28**, 382–391.

Holene, E., Nafstad, I., Skaare, J. U., and Sagvolden, T. (1998). Behavioural hyperactivity in rats following postnatal exposure to sub-toxic doses of polychlorinated biphenyl congeners 153 and 126. *Behav. Brain Res.* **94**, 213–224.

Honma, T., Suda, M., Miyagawa, M., Wang, R. S., Kobayashi, K., and Sekiguchi, S. (2009). Alteration of brain neurotransmitters in female rat offspring induced by prenatal administration of 16 and 64 mg/kg of 2,2',4,4',5,5'-hexachlorobiphenyl (PCB153). *Ind. Health* **47**, 11–21.

Johansson, C., Tofighi, R., Tamm, C., Goldoni, M., Mutti, A., and Ceccatelli, S. (2006). Cell death mechanisms in AtT20 pituitary cells exposed to polychlorinated biphenyls (PCB 126 and PCB 153) and methylmercury. *Toxicol. Lett.* **167**, 183–190.

Johansson, N., Viberg, H., Fredriksson, A., and Eriksson, P. (2008). Neonatal exposure to deca-brominated diphenyl ether (PBDE 209) causes dose–response changes in spontaneous behaviour and cholinergic susceptibility in adult mice. *Neurotoxicology* **29**, 911–919.

Kamata, R., Shiraishi, F., Kageyama, S., and Nakajima, D. (2015). Detection and measurement of the agonistic activities of PCBs and mono-hydroxylated PCBs to the constitutive androstane receptor using a recombinant yeast assay. *Toxicol. In Vitro* **29**, 1859–1867.

Kato, Y., Onishi, M., Haraguchi, K., Ikushiro, S., Ohta, C., Koga, N., Endo, T., Yamada, S., and Degawa, M. (2011). A possible mechanism for 2,2',4,4',5,5'-hexachlorobiphenyl-mediated decrease in serum thyroxine level in mice. *Toxicol. Appl. Pharmacol.* **254**, 48–55.

Kato, Y., Tamaki, S., Haraguchi, K., Ikushiro, S., Sekimoto, M., Ohta, C., Endo, T., Koga, N., Yamada, S., and Degawa, M. (2012). Comparative study on 2,2',4,5,5'-pentachlorobiphenyl-mediated decrease in serum thyroxine level between C57BL/6 and its transthyretin-deficient mice. *Toxicol. Appl. Pharmacol.* **263**, 323–329.

Kato, Y., Haraguchi, K., Ito, Y., Fujii, A., Yamazaki, T., Endo, T., Koga, N., Yamada, S., and Degawa, M. (2010). Polychlorinated biphenyl-mediated decrease in serum thyroxine level in rodents. *Drug Metab. Dispos.* **38**, 697–704.

Kim, K. H., Bose, D. D., Ghogha, A., Riehl, J., Zhang, R., Barnhart, C. D., Lein, P. J., and Pessah, I. N. (2011). *Para*- and *ortho*-substitutions are key determinants of polybrominated diphenyl ether activity toward ryanodine receptors and neurotoxicity. *Environ. Health Perspect.* **119**, 519–526.

Kitamura, S., Jinno, N., Suzuki, T., Sugihara, K., Ohta, S., Kuroki, H., and Fujimoto, N. (2005). Thyroid hormone-like and

estrogenic activity of hydroxylated PCBs in cell culture. *Toxicology* **208**, 377–387.

Kobayashi, K., Miyagawa, M., Wang, R. S., Suda, M., Sekiguchi, S., and Honma, T. (2009). Effects of *in utero* exposure to 2,2',4,4',5,5'-hexachlorobiphenyl on postnatal development and thyroid function in rat offspring. *Ind. Health* **47**, 189–197.

Kodavanti, P. R., Ward, T. R., McKinney, J. D., Waller, C. L., and Tilson, H. A. (1996). Increased [3H]phorbol ester binding in rat cerebellar granule cells and inhibition of 45Ca^{2+} sequestration in rat cerebellum by polychlorinated diphenyl ether congeners and analogs: Structure-activity relationships. *Toxicol. Appl. Pharmacol.* **138**, 251–261.

Kopec, A. K., Burgoon, L. D., Ibrahim-Aibo, D., Mets, B. D., Tashiro, C., Potter, D., Sharratt, B., Harkema, J. R., and Zacharewski, T. R. (2010). PCB153-elicited hepatic responses in the immature, ovariectomized C57BL/6 mice: Comparative toxicogenomic effects of dioxin and non-dioxin-like ligands. *Toxicol. Appl. Pharmacol.* **243**, 359–371.

Kretschmer, X. C., and Baldwin, W. S. (2005). CAR and PXR: Xenosensors of endocrine disrupters? *Chem. Biol. Interact.* **155**, 111–128.

Kuriyama, S. N., Talsness, C. E., Grote, K., and Chahoud, I. (2005). Developmental exposure to low dose PBDE 99: Effects on male fertility and neurobehavior in rat offspring. *Environ. Health Perspect.* **113**, 149–154.

Kuriyama, S. N., Wanner, A., Fidalgo-Neto, A. A., Talsness, C. E., Koerner, W., and Chahoud, I. (2007). Developmental exposure to low-dose PBDE-99: Tissue distribution and thyroid hormone levels. *Toxicology* **242**, 80–90.

Langeveld, W. T., Meijer, M., and Westerink, R. H. S. (2012). Differential effects of 20 non-dioxin-like PCBs on basal and depolarization-evoked intracellular calcium levels in PC12 cells. *Toxicol. Sci.* **126**, 487–496.

Lans, M. C., Spiertz, C., Brouwer, A., and Koeman, J. H. (1994). Different competition of thyroxine binding to transthyretin and thyroxine-binding globulin by hydroxy-PCBs, PCDDs and PCDFs. *Eur. J. Pharmacol.* **270**, 129–136.

Lans, M. C., Klasson-Wehler, E., Willemsen, M., Meussen, E., Safe, S., and Brouwer, A. (1993). Structure-dependent, competitive interaction of hydroxy-polychlorobiphenyls, -dibenzo-*p*-dioxins and -dibenzofurans with human transthyretin. *Chem. Biol. Interact.* **88**, 7–21.

Lee, E., Kim, T. H., Choi, J. S., Nabanata, P., Kim, N. Y., Ahn, M. Y., Jung, K. K., Kang, I. H., Kim, T. S., Kwack, S. J., *et al.* (2010). Evaluation of liver and thyroid toxicity in Sprague-Dawley rats after exposure to polybrominated diphenyl ether BDE-209. *J. Toxicol. Sci.* **35**, 535–545.

Lee, Y. J., and Yang, J. H. (2012). Chlorination of *ortho*-position on polychlorinated biphenyls increases Protein Kinase C activity in neuronal cells. *Toxicol. Res.* **28**, 107–112.

Lesmana, R., Shimokawa, N., Takatsuru, Y., Iwasaki, T., and Koibuchi, N. (2014). Lactational exposure to hydroxylated polychlorinated biphenyl (OH-PCB 106) causes hyperactivity in male rat pups by aberrant increase in dopamine and its receptor. *Environ. Toxicol.* **29**, 876–883.

Li, F., Xie, Q., Li, X., Li, N., Chi, P., Chen, J., Wang, Z., and Hao, C. (2010). Hormone activity of hydroxylated polybrominated diphenyl ethers on human thyroid receptor-beta: *In vitro* and *in silico* investigations. *Environ. Health Perspect.* **118**, 602–606.

Li, T., Wang, W., Pan, Y. W., Xu, L., and Xia, Z. (2013). A hydroxylated metabolite of flame-retardant PBDE-47 decreases the survival, proliferation, and neuronal differentiation of primary cultured adult neural stem cells and interferes with signaling of ERK5 MAP kinase and neurotrophin 3. *Toxicol. Sci.* **134**, 111–124.

Lilienthal, H., Heikkinen, P., Andersson, P. L., van der Ven, L. T., and Viluksela, M. (2014). Dopamine-dependent behavior in adult rats after perinatal exposure to purity-controlled polychlorinated biphenyl congeners (PCB52 and PCB180). *Toxicol. Lett* **224**, 32–39.

Liu, C., Wang, C., Yan, M., Quan, C., Zhou, J., and Yang, K. (2012). PCB153 disrupts thyroid hormone homeostasis by affecting its biosynthesis, biotransformation, feedback regulation, and metabolism. *Horm. Metab. Res.* **44**, 662–669.

Llansola, M., Montoliu, C., Boix, J., and Felipo, V. (2010). Polychlorinated biphenyls PCB 52, PCB 180, and PCB 138 impair the glutamate-nitric oxide-cGMP pathway in cerebellar neurons in culture by different mechanisms. *Chem. Res. Toxicol.* **23**, 813–820.

Marchesini, G. R., Meimaridou, A., Haasnoot, W., Meulenberg, E., Albertus, F., Mizuguchi, M., Takeuchi, M., Irth, H., and Murk, A. J. (2008). Biosensor discovery of thyroxine transport disrupting chemicals. *Toxicol. Appl. Pharmacol.* **232**, 150–160.

Meerts, I. A., van Zanden, J. J., Luijks, E. A., van Leeuwen-Bol, I., Marsh, G., Jakobsson, E., Bergman, A., and Brouwer, A. (2000). Potent competitive interactions of some brominated flame retardants and related compounds with human transthyretin *in vitro*. *Toxicol. Sci.* **56**, 95–104.

Meerts, I. A., Assink, Y., Cenijn, P. H., Van Den Berg, J. H., Weijers, B. M., Bergman, A., Koeman, J. H., and Brouwer, A. (2002). Placental transfer of a hydroxylated polychlorinated biphenyl and effects on fetal and maternal thyroid hormone homeostasis in the rat. *Toxicol. Sci.* **68**, 361–371.

Murk, A. J., Rijntjes, E., Blaauboer, B. J., Clewell, R., Crofton, K. M., Dingemans, M. M., Furlow, J. D., Kavlock, R., Köhrle, J., Opitz, R., *et al.* (2013). Mechanism-based testing strategy using *in vitro* approaches for identification of thyroid hormone disrupting chemicals. *Toxicol. In Vitro* **27**, 1320–1346.

Ness, D. K., Schantz, S. L., Moshtaghian, J., and Hansen, L. G. (1993). Effects of perinatal exposure to specific PCB congeners on thyroid hormone concentrations and thyroid histology in the rat. *Toxicol. Lett.* **68**, 311–323.

Pacyniak, E. K., Cheng, X., Cunningham, M. L., Crofton, K., Klaassen, C. D., and Guo, G. L. (2007). The flame retardants, polybrominated diphenyl ethers, are pregnane X receptor activators. *Toxicol. Sci.* **97**, 94–102.

Pereira, L. C., de Souza, A. O., and Dorta, D. J. (2013). Polybrominated diphenyl ether congener (BDE-100) induces mitochondrial impairment. *Basic Clin. Pharmacol. Toxicol.* **112**, 418–424.

Pessah, I. N., Cherednichenko, G., and Lein, P. J. (2010). Minding the calcium store: Ryanodine receptor activation as a convergent mechanism of PCB toxicity. *Pharmacol. Ther.* **125**, 260–285.

Pessah, I. N., Hansen, L. G., Albertson, T. E., Garner, C. E., Ta, T. A., Do, Z., Kim, K. H., and Wong, P. W. (2006). Structure-activity relationship for noncoplanar polychlorinated biphenyl congeners toward the ryanodine receptor-Ca^{2+} channel complex type 1 (RyR1). *Chem. Res. Toxicol.* **19**, 92–101.

Peters, A. K., van Londen, K., Bergman, A., Bohonowych, J., Denison, M. S., van den Berg, M., and Sanderson, J. T. (2004). Effects of polybrominated diphenyl ethers on basal and TCDD-induced ethoxyresorufin activity and cytochrome P450-1A1 expression in MCF-7, HepG2, and H4IIE cells. *Toxicol. Sci.* **82**, 488–496.

Piedrafita, B., Erceg, S., Cauli, O., and Felipo, V. (2008). Developmental exposure to polychlorinated biphenyls or

methylmercury, but not to its combination, impairs the glutamate-nitric oxide-cyclic GMP pathway and learning in 3-month-old rats. *Neuroscience* **154**, 1408–1416.

Purkey, H. E., Palaninathan, S. K., Kent, K. C., Smith, C., Safe, S. H., Sacchettini, J. C., and Kelly, J. W. (2004). Hydroxylated polychlorinated biphenyls selectively bind transthyretin in blood and inhibit amyloidogenesis: Rationalizing rodent PCB toxicity. *Chem. Biol.* **11**, 1719–1728.

Quinete, N., Schettgen, T., Bertram, J., and Kraus, T. (2014). Occurrence and distribution of PCB metabolites in blood and their potential health effects in humans: A review. *Environ. Sci. Pollut. Res. Int.* **21**, 11951–11972.

Ren, X. M., Guo, L. H., Gao, Y., Zhang, B. T., and Wan, B. (2013). Hydroxylated polybrominated diphenyl ethers exhibit different activities on thyroid hormone receptors depending on their degree of bromination. *Toxicol. Appl. Pharmacol.* **268**, 256–263.

Richardson, V. M., Ferguson, S. S., Sey, Y. M., and Devito, M. J. (2014). *In vitro* metabolism of thyroxine by rat and human hepatocytes. *Xenobiotica* **44**, 391–403.

Richardson, V. M., Staskal, D. F., Ross, D. G., Diliberto, J. J., DeVito, M. J., and Birnbaum, L. S. (2008). Possible mechanisms of thyroid hormone disruption in mice by BDE 47, a major polybrominated diphenyl ether congener. *Toxicol. Appl. Pharmacol.* **226**, 244–250.

Safe, S. (1993). Toxicology, structure-function relationship, and human and environmental health impacts of polychlorinated biphenyls: Progress and problems. *Environ. Health Perspect.* **100**, 259–268.

Schantz, S. L., Moshtaghian, J., and Ness, D. K. (1995). Spatial learning deficits in adult rats exposed to ortho-substituted PCB congeners during gestation and lactation. *Fundam. Appl. Toxicol.* **26**, 117–126.

Schreiber, T., Gassmann, K., Götz, C., Hübenthal, U., Moors, M., Krause, G., Merk, H. F., Nguyen, N. H., Scanlan, T. S., Abel, J., *et al.* (2009). Polybrominated diphenyl ethers induce developmental neurotoxicity in a human *in vitro* model: Evidence for endocrine disruption. *Environ. Health Perspect.* **118**, 572–578.

Schroeder, A. C., and Privalsky, M. L. (2014). Thyroid hormones, t3 and t4, in the brain. *Front. Endocrinol. (Lausanne)* **5**, 40.

Seegal, R. F., Brosch, K. O., and Okoniewski, R. J. (1997). Effects of *in utero* and lactational exposure of the laboratory rat to 2,4,2',4'- and 3,4,3',4'-tetrachlorobiphenyl on dopamine function. *Toxicol. Appl. Pharmacol.* **146**, 95–103.

Shunthirasingham, C., Gawor, A., Hung, H., Brice, K. A., Su, K., Alexandrou, N., Dryfhout-Clark, H., Backus, S., Sverko, E., Shin, C., *et al.* (2016). Atmospheric concentrations and loadings of organochlorine pesticides and polychlorinated biphenyls in the Canadian Great Lakes Basin (GLB): Spatial and temporal analysis (1992-2012). *Environ. Pollut.* Advance Access published February 1, 2016. doi:10.1016/j.envpol.2016.01.039.

Simon, T., Britt, J. K., and James, R. C. (2007). Development of a neurotoxic equivalence scheme of relative potency for assessing the risk of PCB mixtures. *Regul. Toxicol. Pharmacol.* **48**, 148–170.

Sueyoshi, T., Li, L., Wang, H., Moore, R., Kodavanti, P. R., Lehmler, H. J., Negishi, M., and Birnbaum, L. S. (2014). Flame retardant BDE-47 effectively activates nuclear receptor CAR in human primary hepatocytes. *Toxicol. Sci.* **137**, 292–302.

Suvorov, A., Girard, S., Lachapelle, S., Abdelouahab, N., Sebire, G., and Takser, L. (2009). Perinatal exposure to low-dose BDE-47, an emergent environmental contaminant, causes hyperactivity in rat offspring. *Neonatology* **95**, 203–209.

Ta, T. A., Koenig, C. M., Golub, M. S., Pessah, I. N., Qi, L., Aronov, P. A., and Berman, R. F. (2011). Bioaccumulation and behavioral effects of 2,2',4,4'-tetrabromodiphenyl ether (BDE-47) in perinatally exposed mice. *Neurotoxicol. Teratol.* **33**, 393–404.

Tan, Y., Song, R., Lawrence, D., and Carpenter, D. O. (2004). *Ortho*-substituted but not coplanar PCBs rapidly kill cerebellar granule cells. *Toxicol. Sci.* **79**, 147–156.

Tolson, A. H., and Wang, H. (2010). Regulation of drug-metabolizing enzymes by xenobiotic receptors: PXR and CAR. *Adv. Drug Deliv. Rev.* **62**, 1238–1249.

Tseng, L. H., Li, M. H., Tsai, S. S., Lee, C. W., Pan, M. H., Yao, W. J., and Hsu, P. C. (2008). Developmental exposure to decabromodiphenyl ether (PBDE 209): Effects on thyroid hormone and hepatic enzyme activity in male mouse offspring. *Chemosphere* **70**, 640–647.

UNEP (2013). Human exposure to POPs across the globe: POPs levels and human health implications [unep.org]. Accessed July 19, 2016.

Van den Berg, M., Birnbaum, L. S., Denison, M., De Vito, M., Farland, W., Feeley, M., Fiedler, H., Hakansson, H., Hanberg, A., Haws, L., *et al.* (2006). The 2005 World Health Organization reevaluation of human and mammalian toxic equivalency factors for dioxins and dioxin-like compounds. *Toxicol. Sci.* **93**, 223–241.

Viberg, H., Johansson, N., Fredriksson, A., Eriksson, J., Marsh, G., and Eriksson, P. (2006). Neonatal exposure to higher brominated diphenyl ethers, hepta-, octa-, or nonabromodiphenyl ether, impairs spontaneous behavior and learning and memory functions of adult mice. *Toxicol. Sci***92**, 211–218.

Viberg, H., Fredriksson, A., and Eriksson, P. (2003a). Neonatal exposure to polybrominated diphenyl ether (PBDE 153) disrupts spontaneous behaviour, impairs learning and memory, and decreases hippocampal cholinergic receptors in adult mice. *Toxicol. Appl. Pharmacol.* **192**, 95–106.

Viberg, H., Fredriksson, A., and Eriksson, P. (2002). Neonatal exposure to the brominated flame retardant 2,2',4,4',5-pentabromodiphenyl ether causes altered susceptibility in the cholinergic transmitter system in the adult mouse. *Toxicol. Sci.* **67**, 104–107.

Viberg, H., Fredriksson, A., and Eriksson, P. (2004). Neonatal exposure to the brominated flame-retardant, 2,2',4,4',5-pentabromodiphenyl ether, decreases cholinergic nicotinic receptors in hippocampus and affects spontaneous behaviour in the adult mouse. *Environ. Toxicol. Pharmacol.* **17**, 61–65.

Viberg, H., Fredriksson, A., Jakobsson, E., Orn, U., and Eriksson, P. (2003b). Neurobehavioral derangements in adult mice receiving decabrominated diphenyl ether (PBDE 209) during a defined period of neonatal brain development. *Toxicol. Sci.* **76**, 112–120.

Westerink, R. H. S. (2014). Modulation of cell viability, oxidative stress, calcium homeostasis, and voltage- and ligand-gated ion channels as common mechanisms of action of (mixtures of) non-dioxin-like polychlorinated biphenyls and polybrominated diphenyl ethers. *Environ. Sci. Pollut. Res. Int.* **21**, 6373–6383.

Wu, B., Zhang, Y., Kong, J., Zhang, X., and Cheng, S. (2009). *In silico* predication of nuclear hormone receptors for organic pollutants by homology modeling and molecular docking. *Toxicol. Lett.* **191**, 69–73.

Xiong, Y., Ibhazehiebo, K., Iwasaki, T., and Koibuchi, N. (2012). An *in vitro* method to study the effects of thyroid hormone-disrupting chemicals on neuronal development. *Neurotoxicology* **33**, 753–757.

Yan, T., Xiang, L., Xuejun, J., Chengzhi, C., Youbin, Q., Xuelan, Y., Yang, L., Changyan, P., and Hui, C. (2012). Spatial learning and memory deficit of low level polybrominated diphenyl ethers-47 in male adult rat is modulated by intracellular glutamate receptors. *J. Toxicol. Sci.* **37**, 223–233.

Yang, J. M., Salmon, A. G., and Marty, M. A. (2010). Development of TEFs for PCB congeners by using an alternative biomarker–thyroid hormone levels. *Regul. Toxicol. Pharmacol.* **56**, 225–236.

Yang, W., Shen, S., Mu, L., and Yu, H. (2011). Structure-activity relationship study on the binding of PBDEs with thyroxine transport proteins. *Environ. Toxicol. Chem.* **30**, 2431–2439.

Yilmaz, B., Sandal, S., Chen, C. H., and Carpenter, D. O. (2006). Effects of PCB 52 and PCB 77 on cell viability, $[Ca^{2+}]_i$ levels and membrane fluidity in mouse thymocytes. *Toxicology* **217**, 184–193.

Zhang, H., Li, X., Nie, J., and Niu, Q. (2013). Lactation exposure to BDE-153 damages learning and memory, disrupts spontaneous behavior and induces hippocampus neuron death in adult rats. *Brain Res.* **1517**, 44–56.